pre-existing respiratory tract disorders increase risk of developing severe RSV infection. There is conflicting evidence about whether severe RSV disease is correlated to RSV genotype. Molecular epidemiological data from tropical Asia is relatively lacking. This study aims to identify the potential risk factors of severe RSV infection by analysis of clinical data and RSV genotypes in Malaysia.

Methods: The medical records of 138 RSV cases were analysed for demographic and clinical data, including age, gender, ethnicity, prematurity, co-morbidity, and source of infection. Severe RSV infection was defined in patients who died, or required ventilation, intensive care, or inotropes. The hypervariable region of the G gene was sequenced for 91 RSV samples from 1989-2009, for genotype identification and phylogenetic analysis. Demographic factors, clinical factors and genotype were entered into logistic regression analysis as predictors of severe RSV disease. A p-value of <0.05 was considered significant.

Results: The mean age of the study population was 1.4 years, with a majority (62.3%) aged <1 year. Multivariate analysis showed three independent predictors of severe RSV: male gender (odds ratio 4.25; 95% confidence intervals 1.14–15.85), underlying co-morbidity or prematurity (OR 7.11; CI 2.11–23.94), and nosocomial infection (OR 4.71; CI 1.27–17.41). Genotype was not associated with severity. Of the 91 RSV samples sequenced, 66 (72.5%) were in subgroup A and 25 (27.5%) were in subgroup B. The most commonly detected genotypes were GA2 (52.8%, n=48), GB13 (20.9%, n=19), and GA5 (13.2%, n=12). Nucleotide and amino acid similarity between subgroups were 49.9% and 27.0%. RSV A was detected every year, but RSV B only circulated for 2-3 years before being undetected for 1-2 years.

Conclusion: Male gender, underlying co-morbidity or prematurity, and nosocomial infection increases the risk of developing severe RSV infection. RSV A was seen every year, while RSV B circulated in 4-5 year cycles.

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Unsolved epidemiological questions on dengue/dengue hemorrhagic fever– From Taiwan's experiences to global control

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Background: The numbers of dengue hemorrhagic fever (DHF) have been increasing globally in recent three decades. The mechanisms that can explain these epidemiological changes have remained enigma. Through epidemiological field observations, two past major hypotheses for DHF are: (1) primary infection due to viral virulence proposed by Dr. L Rosen and (2) secondary infection of heterologous serotypes of dengue viruses (DENV) with DHF cases whereas primary infection of DENV has frequently identified in Taiwan and other areas without dengue endemicity. Most interestingly, these two hypotheses have not yet fully explained the mechanisms in increasing epidemic severity caused by DENV with high sequence identities over time in the same epidemics (Taiwan's 1998, 2001-2002, 2006 and 2009-2010 epidemics of dengue/DHF) or over years during cross-country spread.

Methods: Viral load, DENV quasispecies and cytokines/chemokines were measured from DF and DHF patients.

Results: We found that Taiwan's DHF cases in later period of epidemics of dengue/DHF in 1998, 2002 and 2006 showed more severe DHF cases. Additionally, DENV obtained from later cases of the same family member showed more virus diversity based on quasispecies analysis and thus leading to higher viral load in DHF than DF patients. Recently, our research team identified key epidemiologic conditions for increasing DHF cases, including longer duration per epidemic wave and higher transmission intensity of dengue cases in areas regardless high or low population density that would be very helpful in global control to minimize numbers of DHF cases. At present, we are investigating viral factors, immuno-attributes and entomologic transmission features with clear epidemiological characteristics and clinical findings in Kaohsiung's DENV-2 epidemic in Taiwan that might explain the increasing clinical and epidemiological severity of DHF over time periods not only in Taiwan but also in Cuba in 1981, 1997 and other South American countries. The preliminary results showed that both innate immunity and adaptive immunity contribute to immune-pathogenesis of DHF cases.

Conclusion: In conclusion, evolution of dengue viruses might happen through epidemics with more influence of immunity at both individual and population levels.

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Frequent in-migration and highly focal transmission of dengue viruses among children in Kamphaeng Phet, Thailand

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Background: Dengue is the leading cause of mosquito-borne viral disease in the world, and dengue fever (DF) and dengue hemorrhagic fever (DHF) continue to increase and geographic range.

There is no effective vaccine availabe to the public currently. Understanding the microevolution of dengue virus (DENV) is central to the deployment of successful intervention strategies.

Methods: We performed a phylogenetic analysis of the envelope (E) genes of DENV-1, -2, and -4 isolates collected from school-based cohort and village-based cluster studies in Kamphaeng Phet, Thailand between 2004 and 2007, with the aim of describing the spatial and temporal patterns of DENV transmission within a rural population where a future vaccine efficacy trial will be conducted.

Results: Our analysis revealed considerable genetic diversity within the study population, with multiple lineages within each serotype circulating throughout the study period, suggesting frequent migration of DENV into this environment, possibly through multiple routes. In contrast, the persistence of viral lineages across sampling years was observed less frequently. Analysis of phylogenetic clustering indicated that DENV transmission is highly spatially and temporally focal, confirming that DENV transmission generally occurs at home rather than at school. The strength of temporal clustering also suggests that the frequency of lineage persistence may be determined by seasonal bottlenecks in the DENV population, which may also facilitate the frequent introduction and establishment of viruses from outside of the study area.

Conclusion: We found the considerable genetic diversity of DENV circulating within a rural population in Kamphaeng Phet, with multiple lineages within each serotype circulating throughout the study period. DENV transmission is highly spatially and temporally focal within this stably endemic area.

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The cells of innate immunity in hemorrhagic fever with renal syndrome

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Background: Hantaviruses (HV) are enveloped, negative-strand RNA viruses of the Bunyaviridae family which can be lethal for human causing a hemorrhagic fever with renal syndrome (HFRS). The innate response occurs within minutes of infection and includes many factors of protection of an organism in particular phagocyte – neutrophils and monocytes/macrophages. These cells are keys in innate immunity, but their role in viral pathogenesis is incompletely understood.

Methods: The monocytes were obtained by Ficoll-Hypaque gradient centrifugation of peripheral blood, macrophages and neutrophils were collected by washing the peritoneal cavity of guinea pigs. The Hantaan-like strain 308 isolated from Apodemus agrarius, adapted previously to cells Vero E6, was used for infected cells. The cells were stained with indicated antibodies to viral proteins followed by Alexafluore 546 conjugated secondary antibodies. Slides were examined by LSM510META multiphoton confocal laser scanning microscope and scanning (SEM) electron microscopy (Ultra55, Zeiss). The activities of enzymes plasmalemma (ATPase and 5'-nucleotidase), enzymes of oxide-dependent systems and quantity of nitrite ions in infected cells were determined.

Results: It was established that after 30-minute contact of HV with cells 45.5 ± 4.6% phagocytes became antigen-positive. The adhesion of HV on phagocytes was confirmed by titration of supernatant virus-containing fluids on the cell culture. It was defined that after 60 min of infection the reduction in titer of 2.5 log units was determined. The susceptibility of monocyte-derived cells for HV infection increased when their achievement of final stage of differentiation. With the help of SEM the ability of HV to adhere for surface of neutrophils was revealed and the specific features of cell stimulation was determined.

Conclusion: The reduction of the enzymes 5 '-nucleotidase and ATPase activity was detected on the background of the maximum number of antigen-positive cells. The activation of nitric oxide-dependent mechanism was revealed at earlier period, than activation of oxide-dependent systems of cells. Also in the first hour of HV infection the intracellular contents of lysosomal enzymes was increased. Thus, our results showed the increased metabolic activity of macrophage and neutrophils in process of HV infection, which suggests the important role of these cells of innate immunity in the pathogenesis of HFRS.

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Cytomegalovirus evolution in congenitally infected twins

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Background: Cytomegalovirus (CMV) is genetically the most complex viral pathogen of humans and the leading cause of infection-associated birth defects. CMV, unlike most viruses, is able to cross the placenta during pregnancy and establish a lifelong infection in the fetus. The viral population dynamics associated with this route of infection are poorly understood.

Methods: We present a study of CMV genomic populations sampled from the urine of congenitally infected, monozygotic twins at 1, 2, and 11 months of age. Virion dsDNA was deep sequenced as described (Renzette, et al., 2011. PLoS Pathogens 7(5):e1001344) for high-resolution population genetic modeling and analyses. An average of ~1,700 genomes/sample were sequenced and analyzed.

Results: The CMV populations exhibit nucleotide and amino acid diversity scores comparable to those of RNA virus populations. The 1 and 2 month samples reveal generally stable population structures across time and between hosts. The 11-month time point shows significant increases in intrapopulation diversity and interpopulation differentiation as measured by nucleotide diversity and FST, respectively. A maximum likelihood estimate of initial infection is 16.5 weeks gestational age (95% CI: 15.9 – 17.1 weeks), which is in agreement with previous estimates based on patterns of maternal seroconversion data. We find evidence for three sequential population bottlenecks and expansions in both twins, though their magnitudes differ between the hosts. The timing of these events is consistent with population bottlenecks and expansions that would occur during viral colonization of the placenta, fetal blood, and fetal kidney. Interestingly, there is strong evidence